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**Advanced Methods of  
Pharmacokinetic and  
Pharmacodynamic  
Systems Analysis  
Volume 2**

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**Plenum Press • New York and London**

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## BUILDING POPULATION PHARMACOKINETIC- PHARMACODYNAMIC MODELS

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### INTRODUCTION

One of the major tasks in clinical pharmacology is to estimate population characteristics, such as the mean and inter-individual variability of pharmacokinetic (PK) and pharmacodynamic (PD) parameters in a patient population. Population analysis techniques try to quantify this variability within the population and account for it in terms of patient specific covariates such as age, weight, disease state, concurrent therapy and others. The derived population PK or PD model provides the necessary information required to optimize individual dose: for initial dose, the population mean PK and PD parameters and their relationships with patient specific covariates suffice; for subsequent dose adjustment, the probability distribution of inter-individual random effects which capture inter-individual differences that are not explicable by patient specific covariates; and the residual variability due to measurement errors and intra-individual variability can be used, along with observed individual responses to the initial dose, to arrive at an adjusted dose.

Population analysis according to (nonlinear) mixed effects models, as a means of obtaining population PK-PD parameter estimates, has gained considerable popularity during the past decade because it permits PK-PD characterization of actual patient populations. It permits such characterization because it can make use of only a few samples from each individual collected during routine clinical care or as a supplement to a study designed for other purposes. Certain patient groups,

especially the elderly, the critically ill, and children may be hard to study under the rigorous conditions of well controlled clinical trials, and for them, sparse sample based methods may be the only practical way to determine population PK-PD.

The discovery of a population model that adequately describes a given data set is often a complicated and time consuming task. This is partly due to the fact that the relationships of greatest interest, those between PK or PD parameters and covariates, are not directly observed; only the consequences of those relationships, concentrations or effects vs time, are observed. The modeling task consists not only in identifying those covariates (patient specific characteristics) that significantly influence the PK or PD parameters but also consists in characterizing the shape of the relationships between covariates and parameters. In a previous paper [1] we presented some model-building techniques that simplify this task. In this paper we review that previous work, and also extend it. We present here methods that, (i) efficiently find and depict relationships between PK or PD parameters and patient covariates when they exist, (ii) screen for potential interactions between covariates, and (iii) provide diagnostics for detecting individuals that have an unusually large influence on the choice of covariates or shape of relationships. While the NONMEM program [2,3] is used for the work reported here, the ideas and techniques we discuss are general and are easily transferred to other nonlinear mixed effects analysis platforms.

## BASIC POPULATION MODEL

The population model has two parts: (i) a model for the response of an individual within the population (individual model) and (ii) a model relating the individual PK-PD parameters to observable patient characteristics (population model).

The  $j$ th measurement (e.g., plasma concentration or effect) for the  $i$ th individual can be related to the PK or PD parameters in the following way (individual model)

$$y_{ij} = f(\phi_i, x_{ij}) + \epsilon_{ij} \quad (1)$$

where  $f$  is a function (PK-PD model) describing the expected value of the response for a given parameter vector  $\phi_i$  as a function of the covariate values  $x_{ij}$ .  $\phi_i$  is the vector of the model parameters (such as  $Cl$ ,  $EC_{50}$ , etc.) for the  $i$ th individual and  $x_{ij}$  is the set of known covariates for this individual (such as dose, time, etc.). The term  $\epsilon_{ij}$  accounts for the (random) error between the true value and the corresponding measurement. It is generally assumed that  $\epsilon_{ij}$  are independently normally distributed with either a constant variance  $\sigma^2$  for all observations (additive residual error model) or with a variance  $\sigma^2 f(\phi_i, x_{ij})^2$  that is a function of the predicted drug concentration or effect measurement (proportional residual error model).

An important part of variability in pharmacokinetic and pharmacodynamic experiments is due to differences between patients. The individual PK-PD parameters can be related to observable patient characteristics in the following way (population model)

$$\phi_i = g(\theta, z_i) + \eta_i \quad (2)$$

where  $g$  is a known function that describes the expected value of  $\phi_i$  as a function of known individual specific covariates  $z_i$ , such as age, weight, disease states, etc., and the vector of population average parameters  $\theta$ . The vector  $\eta_i$  express the random

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ed value of  $\phi_i$  as a function of eight, disease states, etc., and vector  $\eta_i$  express the random

difference between the population predictions and individual PK-PD parameters. It is assumed that  $\eta_i$  are independently, and multivariately normally distributed with mean zero and variance-covariance matrix  $\Omega$  (diagonal elements denoted  $\omega_k^2$  where  $k$  denotes the  $k$ th parameter). Frequently, an exponential inter-individual error model is used which prescribes a log-normal distribution for the PK-PD parameters

$$\phi_i = g(\theta, z_i) \cdot \exp(\eta_i) \quad (3)$$

in which case  $\omega_k$  is an approximate coefficient of variation.

## DATA

Data on four drugs were used to illustrate our model building and evaluation methods (these are the same data sets used in our previous report [1]). The analysis reported here for these drugs are not meant to be definitive but are presented only to illustrate the procedures which are the subject of this paper.

The first drug is ibuprofen, a nonsteroidal anti-inflammatory. Ibuprofen was administered as a single oral dose of 5 mg/kg or 10 mg/kg to 92 children with febrile illness. Two to 6 plasma samples were obtained from each individual up to 8 hours post dosing (total of 411 plasma samples). The following demographic data were recorded: gender (SEX), male (53), female (40); race (RACE), caucasian (17), black (76); location (LOC), clinic (65), hospital/inpatient (28); food since dosing (FED), no (25), yes (68); dose level (DRG), 5 mg/kg (45), 10 mg/kg (48); height (HT), 64-148 cm; weight (WT), 5.6-45.5 kg; age (AGE), 3-132 months. These data were originally reported in [4], and were kindly supplied by Bristol Myers, Hillside, New Jersey.

The second drug is the antiarrhythmic quinidine, administered orally to 136 hospitalized men for various arrhythmias. Plasma samples were obtained for routine clinical purposes (total of 361 samples). The following demographic data were recorded: race (RACE), caucasian (91), latin (35), black (10); smoking (SMO), no (91), yes (45); ethanol abuse (ET), no ethanol abuse or social drinker (90), current ethanol abuse (16), history of ethanol abuse (30); congestive heart failure (HF), no or mild (56), moderate (40), severe (40); creatinine clearance (RF), < 50 ml/min (48), > 50 ml/min (88); weight (WT), 41-119 kg; height (HT), 154-202 cm; age (AGE), 42-92 years;  $\alpha$ -1-acid glycoprotein concentration (GLP), 39-316 mg/dl. These data were originally reported in [5], and were kindly supplied by Thomas M. Ludden.

The third drug is the antihypertensive prazosin, administered twice daily in a dose range of 1 mg per day up to 20 mg per day in patients with mild, moderate or severe hypertension. Each patient started with the lowest dose and the dose was increased until the patient's arterial pressure was controlled. After at least a period of eight weeks continuous therapy the PKs of prazosin were studied in 64 patients during a 12 hr dosing interval. After 4 weeks, 35 patients were restudied. A total number of 887 plasma samples were obtained. The following demographic data were recorded: gender (SEX), male (63), female (36); race (RACE), caucasian (67), black (32); visit number (VIS), visit 15 (53), visit 16 (46); smoking (TOB), no (74), yes (25); prior therapy (PT), no (3), yes (96); co-treatment with hydrochlorothiazide (HCTZ), no (44), yes (55); co-treatment with propranolol (PROP), no (85), yes (14); other concomitant therapy (CON), no (24), yes (75); food (FF), fed (50), fasting (49); height (HT), 140-188 cm; weight (WT), 51-139 kg; age (AGE), 24-69 years; serum creatinine (SECR), 0.6-1.8 mg/dl. These data were originally reported in [6], and were kindly supplied by Eli Lilly, Indianapolis, Indiana.

The last drug is the broad spectrum antibiotic pefloxacin. The PK of pefloxacin were studied in 74 critically ill patients with infections with organisms sensitive to pefloxacin. The drug was administered twice daily, 200 mg or 400 mg, in 1 hr infusions. For each individual three plasma samples were obtained during a 12 hr dosing interval. In total 113 dosing intervals were studied (337 plasma concentrations). The following demographic data were recorded: age (AGE), 18–84 years; weight (WT), 43–125 kg; creatinine clearance (CLCR), 0.4–312 mL/min; Glasgow score (GLAS), 3–15; simplified acute physiology score (SAPS), 1–26; albumin (ALB), 17–40 g/L; bilirubin (BIL), 4–150  $\mu$ mol/L; alanine amino transferase (ALAT), 3–200 IU/L; alkaline phosphatase (AP), 32–615 IU/L; prothrombin level (PT), 36–100 % normal; systolic blood pressure (SPB), 60–175 mmHg; heart rate (HR), 60–170 beats/min; artificial ventilation (AV), no (42), yes (71); gender (SEX), male (86), female (27); center where study was performed (CEN), center 1 (44), center 2 (69); administration of high dose catecholamine (CAT), no (92), yes (21). These data were originally reported in [7], and were kindly supplied by Rhone Poulenc Rorer, Antony, France.

## MODEL BUILDING PROCEDURE

The population model characterizing the relationships between patient specific characteristics (covariates) and PK-PD parameters is derived using the stepwise approach previously described [1]. Briefly, in a first step individual empirical Bayes estimates of PK-PD parameters are obtained based on a prior NONMEM fit using no covariates. Subsequently, the relationship between the individual PK-PD parameter estimates and candidate covariates is evaluated outside NONMEM using a flexible semi-parametric regression model: the generalized additive model (GAM). Finally, the population analysis is completed using NONMEM on the basis of the GAM analysis of the previous step. This procedure is an extension of the graphical method proposed by Maitre et al. [8]. The model building procedure described above is demonstrated in full in this paper using ibuprofen. The results for the other drugs are used to address specific issues and to evaluate the robustness of the approach.

## STRUCTURAL PK-PD MODEL

In the first step of the analysis the structural PK-PD model without any covariates is derived and fitted to the data. For ibuprofen, using NONMEM (version 4, level 1.0), the time profile of plasma concentrations is best described using a one-compartment model with first order absorption (ADVAN2, see Fig. 1). The model is parameterized in clearance (Cl), volume of distribution (V) and the first order absorption rate constant  $k_a$ . An exponential error model is used to characterize the inter-individual variability in the PK parameters. A proportional error model is used for the residual variability. The values of the population parameters  $\theta$ ,  $\sigma^2$ , and  $\Omega$  are estimated using the so-called first order method in NONMEM. Subsequently, these parameter values are used to obtain empirical Bayes estimates of the individual PK parameters  $\phi_i$  using the POSTHOC step in NONMEM [9,10].

## COVARIATE MODELS

In the second step a regression model is derived that describes the dependence of the individual PK-PD parameter estimates (i.e., the elements of  $\phi_i$ ) on the candidate covariates  $z_{1i} \dots z_{Ni}$ . With the estimates of  $\phi_i$  from the Bayes estimation step treated

Fig. 1. Time profile of plasma concentrations for a patient fitted with a one-compartment PK-model with first order absorption (ADVAN2).

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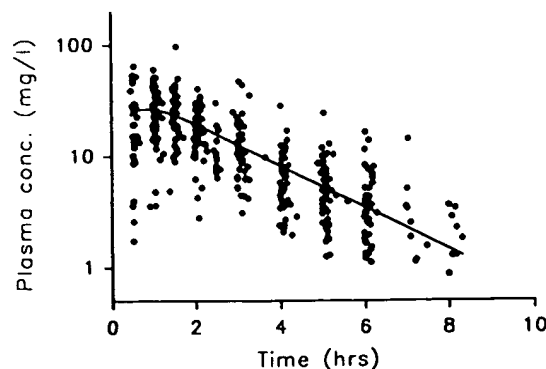


Fig. 1. Time profile of ibuprofen plasma concentrations. The solid line shows the fit of the structural PK-model without any covariates (one-compartment model with first order absorption) using NONMEM to the actual plasma concentrations (•) obtained from 92 children with febrile illness. The plasma concentrations were normalized for a dose of 100 mg.

as data, this step corresponds to the classical regression problem of variable selection and we take advantage of the recent work done on this problem by others [11]. The relationship between covariates and a specific model parameter can in the most general case be described by a multidimensional smooth function  $g(\cdot)$

$$\phi_i = g(z_1, \dots, z_{Ni}) + \eta_i \quad (4)$$

Without some constraints, such a general description of the data is non-identifiable, and even if identifiable, would be difficult to interpret in dimensions higher than two. Furthermore, the number of points required to fill a space to a given density grows exponentially with the dimension of the space, but usually data do not. This makes the estimation of complex multivariate functions using sparse noisy data highly inaccurate. A multiple linear regression model is an often used simplification

$$\phi_i = \theta_0 + \sum_{n=1}^N \theta_n z_{ni} + \eta_i \quad (5)$$

However, this model makes a strong assumption, often unjustifiable on scientific grounds, that a linear relationship exists between parameters and covariates. A more general approach uses a generalized additive model (GAM) [11,12]

$$\phi_i = \theta_0 + \sum_{n=1}^N g_n(z_{ni}) + \eta_i \quad (6)$$

where  $g_n(\cdot)$  is an arbitrary univariate function. The use of splines for  $g_n(\cdot)$  is very appealing because splines are both flexible and convenient to use. (In this paper, wherever splines are mentioned, it is understood that we refer to a natural cubic spline with two internal knots at the 33% and 66% quantiles of the argument variable, equivalent to 4 degrees of freedom [13].) Because of the additive structure of a GAM, the interpretation of the analysis is straightforward, and the independent role of the various covariates can easily be displayed graphically.

Table I. Change in residual sum of squares ( $\Delta$ RSS) when the individual (posterior Bayes) clearance estimates of ibuprofen are regressed independently on each covariate.

| covariate | linear vs. constant |       | spline vs. linear |      |
|-----------|---------------------|-------|-------------------|------|
|           | $\Delta$ RSS        | p     | $\Delta$ RSS      | p    |
| WT        | -3.89               | <0.01 | -0.10             | 0.57 |
| AGE       | -3.88               | <0.01 | -0.13             | 0.49 |
| HT        | -3.64               | <0.01 | -0.21             | 0.33 |
| FED       | -0.61               | 0.03  |                   |      |
| DRG       | -0.58               | 0.03  |                   |      |
| RACE      | -0.46               | 0.06  |                   |      |
| LOC       | -0.07               | 0.45  |                   |      |
| SEX       | -0.03               | 0.60  |                   |      |

## INITIAL SCREENING

As a preliminary screening step before the GAM analysis, the individual PK parameter estimates are regressed independently on each covariate. This step is equivalent to the method proposed by Maitre et al. [8] and may give a first impression of the relative importance of each covariate and of the shape of the relationship between it and the model parameters. The results of this screening step for ibuprofen, with respect only to the parameter clearance, are summarized in Table I. The covariate relationships are modeled linearly or with splines. The significance (p) of the linear vs constant model and the spline vs linear model is tested using the F test. The initial screening shows that AGE, WT, HT, DRG, and FED may significantly ( $p < 0.05$ ) influence the clearance of ibuprofen and that the relationship between the continuous covariates and clearance is best described by a linear model.

## GENERALIZED ADDITIVE MODEL

The generalized additive model is derived using a step-wise addition/deletion method [12]. This method steps through a series of models according to the following algorithm. At each step of the model building each of the covariates is allowed to be deleted from the model, or to enter the model in any of several prespecified functional representations; e.g., linearly or as a spline. At each step the model is advanced by addition, deletion, or replacement of the single additive term that results in the largest decrease in the Akaike Information Criterion (AIC). (The AIC is proportional to the residual sum of squares from the GAM fit plus a penalty, proportional to the number of parameters in the model.) The search stops when the AIC has reached a minimum value. We estimate the GAM using the statistical programming environment S-plus (version 3.0, Statistical Sciences Inc., Seattle, WA). However, a FORTRAN program to perform the GAM analysis is also available (ref. 11, page 307).

A GAM was derived for all PK parameters of ibuprofen;  $Cl$ ,  $V$ , and  $k_a$ , however only the results for  $Cl$  are shown as an example. For ibuprofen the GAM analysis indicated that  $Cl$  is a linear function of WT and a function of the categorical covariates DRG and RACE. The GAM was derived using both a forward search, starting with the NULL model and a backward search, starting with the FULL model, which includes all covariates. A total of 71 different models were tested for  $Cl$ . The path taken to the final GAM is summarized in Table II. This table also shows some of the

Table II. The top part of (CI) of ibuprofen, starting the largest decrease in shows some models cl. the notation of the model figuratively and not lit

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The relationships between shown in Fig. 2. The solid of each of the terms in the plotted represents the cov.  $Cl$  estimates minus the pr shown.

## NONMEM ANALYSIS

In the final (NONMEM) population model is derived. Two strategies (roughly cation) can be used in the N all covariates (with associa the lowest AIC value, and redundant covariates; or (i and attempt, thereafter, to variates appearing in mod covariates can be tested in prefer the second approach large. The parameter esti for the NONMEM analysis: objective function,  $-2 \log$  likelihood reduced model is asymptot

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lysis, the individual PK covariate. This step is to give a first impression of the relationship between the covariate and the response. The first step for ibuprofen, shown in Table I. The covariate significance (p) of the linear model is tested using the F test. The model may significantly (p < 0.05) differ from the linear model.

step-wise addition/deletion according to the following criteria: a covariate is allowed to be added if the model is advanced by at least one step that results in the largest decrease in the AIC. The AIC is proportional to the number of parameters and the log likelihood. The model has reached a minimum when the log likelihood environment S-plus or, a FORTRAN program (307).

for  $Cl$ ,  $V$ , and  $k_a$ , however, the GAM analysis of the categorical covariates was performed by forward search, starting with the FULL model, which was tested for  $Cl$ . The path also shows some of the

Table II. The top part of the table shows the path taken to the final GAM for the clearance ( $Cl$ ) of ibuprofen, starting with the NULL model. In each step the term that results in the largest decrease in the AIC is added (+) to the model. The bottom part of this table shows some models close to the final GAM (model with smallest AIC value). The ~ in the notation of the models means "is a function of" and the + signs are to be interpreted figuratively and not literally.

| step | term     | $\Delta$ RSS | RSS   | AIC   |
|------|----------|--------------|-------|-------|
| 1    | constant | 12.16        | 234.3 |       |
| 2    | +WT      | -3.89        | 8.26  | 200.5 |
| 3    | +DRG     | -0.36        | 7.90  | 198.2 |
| 4    | +RACE    | -0.32        | 7.58  | 196.3 |

Summary of model close to the minimum AIC model

| Equation                        | AIC   |
|---------------------------------|-------|
| $Cl \sim WT + DRG + RACE$       | 196.3 |
| $Cl \sim WT + DRG + RACE + LOC$ | 197.3 |
| $Cl \sim WT + DRG + RACE + SEX$ | 197.3 |

models that (based on the AIC) are very close to the model that best describes the data. Table II shows that WT has the greatest influence on  $Cl$ , explaining 32% of the observed variability in  $Cl$ . DRG and RACE are not as important, and only result in a small additional change of the residual sum of squares, explaining an additional 6% of the variability in  $Cl$ . HT and AGE which appeared to be important covariates according to the initial screening do not appear in the GAM. The reason for this is that these two covariates are highly correlated with WT ( $r > 0.940$ ).

The relationships between covariates and ibuprofen  $Cl$  for the final GAM are shown in Fig. 2. The solid lines displayed in this figure are the additive contributions of each of the terms in the GAM, i.e., the  $g(\cdot)$  for each of the covariates. The actual data plotted represents the covariate vs partial residual values. The latter is the individual  $Cl$  estimates minus the predictions based on all other covariates apart from the one shown.

## NONMEM ANALYSIS

In the final (NONMEM) step of the model-building, the non-linear mixed effect population model is derived, based on the regression models found in the GAM step. Two strategies (roughly corresponding to backward elimination and forward addition) can be used in the NONMEM step: (i) include in the initial NONMEM model all covariates (with associated functional relationships) that appear in the GAM with the lowest AIC value, and in models close to it, and attempt, thereafter, to eliminate redundant covariates; or (ii) start with the final GAM as the initial NONMEM model and attempt, thereafter, to add non-redundant covariates, selecting them from covariates appearing in models close to the final GAM (see e.g. Table II). The potential covariates can be tested in the order of their importance in the GAM analysis. We prefer the second approach, especially when the number of candidate covariates is large. The parameter estimates of the GAM analysis are used as initial estimates for the NONMEM analysis. Model selection is done on the basis of the NONMEM objective function,  $-2 \log$  likelihood ( $-2LL$ ). The difference in  $-2LL$  between a full and reduced model is asymptotically  $\chi^2$  distributed with degrees of freedom equal to the



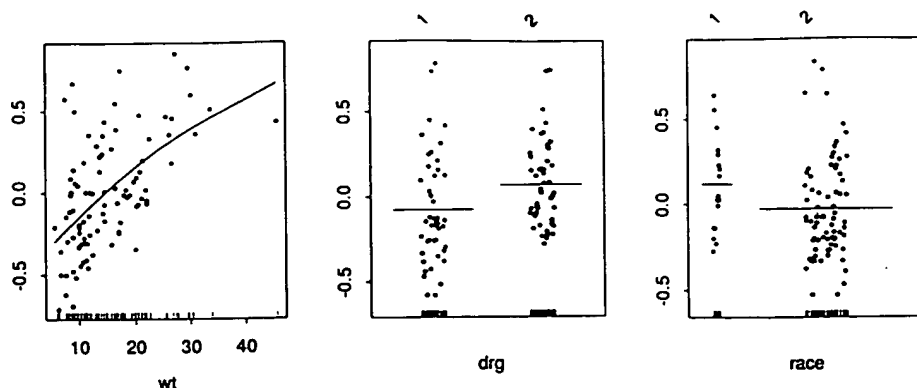


Fig. 2. Partial residual plot of each of the additive terms of the final GAM for the CI of ibuprofen. The solid line in each plot shows the contribution of a term to the additive predictor of CI. The actual data in each of the plots are the partial residuals; i.e., the observed individual CI values minus the prediction based on all terms apart from the one shown. Each curve has been centered to have an average value of zero. The same scale is used for the y-axis in each plot so that the relative importance of the covariates can be compared. The left panel shows the relationship between WT and CI. The solid line represents the contribution to the GAM, characterized by a natural cubic spline with two internal knots at the 33% and 66% quantiles: 11 kg and 17 kg. The middle panel shows the effect of the two dose level groups (DRG, 1= 5 mg/kg and 2= 10 mg/kg). The right panel shows the effect of race (RACE, 1=caucasian, 2=black). For these categorical covariates the points are randomly shifted by small amounts on the abscissa to display them better.

Table III. Change in  $-2LL$  when each of the parameters of the covariates appearing in the final NONMEM model for ibuprofen is set to zero.

| Covariate                       | WT    | DRG  | RACE |
|---------------------------------|-------|------|------|
| $-2\Delta LL$ (vs. $\theta=0$ ) | 115.0 | 16.0 | 9.9  |

difference in number of parameters between the two models. Covariates are added to the model if they significantly decrease the  $-2LL$  ( $> 6.6$ ;  $p < .01$ ), or deleted from the model if  $-2LL$  does not increase significantly. Covariates are also deleted from the model if  $\pm 1$  (asymptotic) SE of the parameter estimate includes zero or when the magnitude of the covariate effect is small on clinical grounds.

The NONMEM step in the ibuprofen model building exercise results in the following population model for CI

$$CI = (\theta_1 + \theta_2 (WT - 15) + \theta_3 DRG + \theta_4 RACE) \cdot \exp(\eta_{CI}) \quad (7)$$

Table III summarizes the change in  $-2LL$  if each of the  $\theta_i$  in the final NONMEM model is set to zero, showing the significance of each of the parameters. Including the final set of covariates in the NONMEM model resulted in a decrease of the inter-individual variability from 46% to 29% for CI. No additional covariate results in a significant change in  $-2LL$ .

Table III also shows that the GAM analysis not only provided all the important covariates for the final NONMEM model, but also that it correctly identified the relative (order of) importance of each (compare Table III and Table II), and the correct shape of the relationship. To check the latter, the relationship of WT to ibuprofen CI (the most important relationship) was modeled in NONMEM with a spline. This

Table IV. Summary of covariates to the GAM and NONMEM analysis, order of appearance in the GAM for ibuprofen, 9 for quinidine, 13 for

| ANALYSIS   | COVARIATES |     |  |
|------------|------------|-----|--|
| Ibuprofen  |            |     |  |
| GAM        | WT         | DRG |  |
| NONMEM     | WT         | DRG |  |
| Quinidine  |            |     |  |
| GAM        | GLP        | WT  |  |
| NONMEM     | GLP        | WT  |  |
| Prazosin   |            |     |  |
| GAM        | HT         | SEX |  |
| NONMEM     | HT         |     |  |
| Pefloxacin |            |     |  |
| GAM        | CICR       | BIL |  |
| NONMEM     | CICR       | BIL |  |

nonparametric representation did confirm the results from the GAM from a linear one.

Similar results were found for LOC, and DRG) and their function final NONMEM model. One additional final GAM model was found to significant. However, this covariate does appear in the model.

## VALUE OF THE GAM STEP

To decide if the GAM step is what it is designed to do, i.e., of their functional relationships to a simpler approach. Our impression

Regarding achieving its goal, the GAM analysis to significantly select all the important covariates in the AIC model. The latter case happened in the model close to the minimum model. The covariates that were selected by the GAM analysis also resulted in significant in the step. Conversely, those covariates that were not selected by the GAM analysis are of minor significance



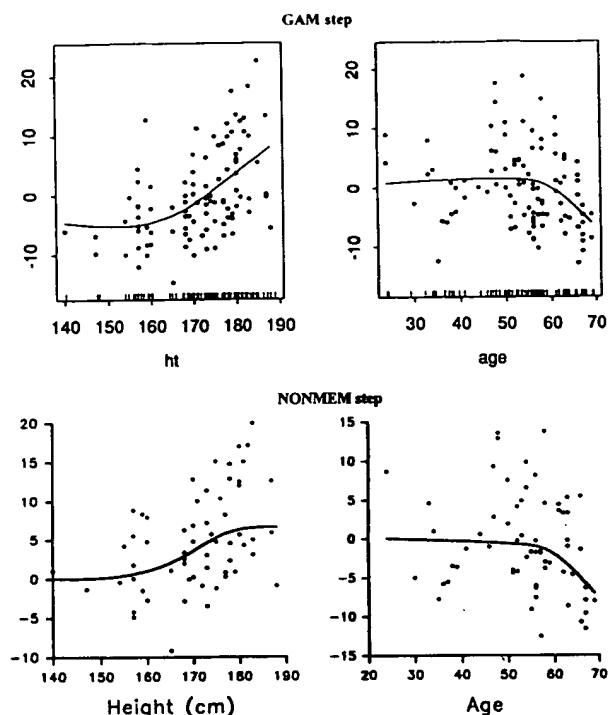


Fig. 3. Partial residual plots for the contribution of HT and AGE to the final GAM for the clearance of prazosin (upper panels) and for the contribution of HT and AGE to the additive NONMEM model (lower panels). The relationships are described by a natural cubic spline with two internal knots at the 33% and 66% quantiles: 168 cm and 177 cm for HT and 52 and 58 years for age. Note that the GAM plots are shifted on the y-axis in comparison to the NONMEM plots because they are centered to have an average of zero. The NONMEM plots show zero effect at the lowest values for HT and AGE. See legend to Figure 2 for further details.

were found for V. So far we have observed that the GAM analysis is inclusive. For all four drugs a total of 27 covariates were found to be of significance in the NONMEM models. Of these covariates, 25 were found in the final GAM. The other 2 were found in models very close to the minimum. Regarding the shape of the functional relationships, prazosin and pefloxacin provide examples. For the former, the GAM analysis identifies important nonlinearities in the relationship between Cl and the covariates HT and AGE. The spline contribution of each of these covariates to the GAM is shown in Fig. 3. A similar nonlinear relationship was included in the NONMEM model, yielding a significant decrease (30.0 points) in  $-2LL$  (4 additional parameters) when compared to a linear model for both covariates. The spline relationships found in the NONMEM step are also shown in Fig. 3. Note the similarity between the splines found in the GAM and NONMEM steps. For pefloxacin the GAM analysis indicates a nonlinear relationship between Cl and WT. A similar relationship is found in the NONMEM step, resulting in a significantly better fit than with the linear model.

Regarding whether the GAM analysis provides a better starting point than a simpler approach, we compared it to the one-covariate-at-a-time initial screening step. For pefloxacin 13 out of the 16 covariates are found to significantly affect Cl according to the one-covariate-at-a-time analysis ( $p < 0.05$ ). The GAM analysis

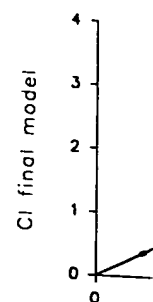


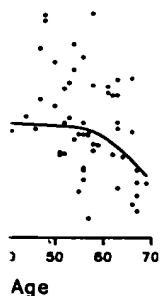
Fig. 4. Plot of empirical Bayes estimates from the basic PK-model without any covariate effects (y-axis). The solid line represents the model's estimate.

narrows this down to 8 covariates for analysis. One of the covariates that was not selected by the one-covariate-at-a-time analysis is HT. Similar results are found for the time analysis tends to select numerous covariates. Of greater importance, the time analysis disregards some of the covariates in the final NONMEM model. The results are influenced by other covariate or by collinearities.

These results show that the GAM analysis provides a better starting point for the NONMEM analysis. The initial model for the NONMEM analysis derived from the GAM model is considered a good starting point for the NONMEM analysis and provides a nice graphical representation of the covariates and model parameters.

## DISCREPANCIES BETWEEN GAM AND NONMEM

There are several factors that may depend on the quality of the data. These estimates tend to be "smooth" and therefore, fail to reveal important nonlinearities detected in the NONMEM step, it is not clear here. One would predict that it might be imprecise or few in number. (ii) The model is built, because the prior distribution of the empirical Bayes estimates of ibuprofen Cl changes from that of the basic PK-model (no covariates) to that of the final NONMEM model. This might prove more of a problem, or sparse. (iii) The individual PK-models from the GAM analysis, but not in the NONMEM analysis, the precision of the empirical Bayes estimates. However, we have not found this to be a problem for one individual from day to day, then



to the final GAM for the clearance  
to the additive NONMEM model  
spline with two internal knots at  
and 58 years for age. Note that the  
IM plots because they are centered  
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GAM analysis is inclusive.  
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und to significantly affect Cl  
< 0.05). The GAM analysis

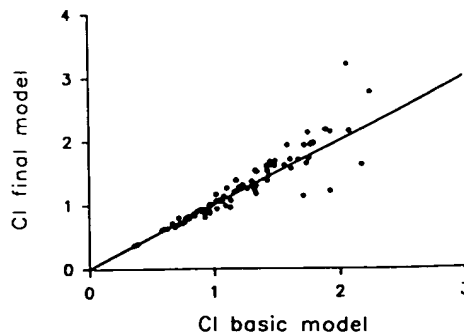


Fig. 4. Plot of empirical Bayes estimates of the individual clearance (Cl) values for ibuprofen obtained from the basic PK-model without any covariates (x-axis) and the final population model including all covariate effects (y-axis). The solid line is the line of unity.

narrows this down to 8 covariates of which 6 are finally selected by the NONMEM analysis. One of the covariates that is selected by the NONMEM analysis, SPB, is not selected by the one-covariate-at-a-time analysis, but does appear in the GAM. Similar results are found for the other drugs. In general, the one-covariate-at-a-time analysis tends to select numerous covariates that are not significant in the final NONMEM analysis, probably because they are collinear with one or a combination of other covariates. Of greater importance, perhaps, is that the one-covariate-at-a-time analysis disregards some of the covariates that appear to be of importance in the final NONMEM model. The reason may be that their effect is masked by some other covariate or by collinearities among covariates.

These results show that the GAM analysis is a robust approach to select a good initial model for the NONMEM analysis. The stepwise addition/deletion method to derive the GAM model is considerably quicker than a similar model search within NONMEM and provides a nice graphical representation of the relationships between covariates and model parameters.

## DISCREPANCIES BETWEEN GAM AND NONMEM STEPS

There are several factors that may influence the success of the GAM step: (i) It may depend on the quality of the empirical Bayes estimates of the PK-PD parameters. These estimates tend to be "shrunk" towards the population mean, and may, therefore, fail to reveal important relationships. To the extent that this problem can be detected in the NONMEM step, it appears not to be present in the data sets analyzed here. One would predict that it might appear when individual data are particularly imprecise or few in number. (ii) The empirical Bayes estimates will change as the model is built, because the prior changes. For the four examples presented here, we have not found this to be a problem. Figure 4 shows that the individual posterior Bayes estimates of ibuprofen Cl change only marginally when the prior distribution is changed from that of the basic NONMEM fit (just the structural model without covariates) to that of the final NONMEM fit (including all covariates). As for the previous problem, this might prove more important when individual data are imprecise or sparse. (iii) The individual PK-PD parameter estimates are weighted equally in the GAM analysis, but not in the NONMEM analysis. This could be solved by using the precision of the empirical Bayes estimates as weights in the GAM analysis. However, we have not found this to be a problem; (iv) The covariates may change within one individual from day to day, thereby obscuring covariate-parameter relationships

80

solved by dividing each indi-  
each of which the covariates  
ccessfully to the prazosin and  
ng to detect within-individual  
e exacerbated as before if data  
data in each "period" to allow  
AIC is used as the selection cri-  
d test is used in the NONMEM  
the operating conditions of our  
f analysis is inclusive, which is

AM is that interactions between  
account, the general structure  
by equation (4) could be used.  
ms discussed earlier. Recently,  
covariate selection [14]. This  
riates showing important inter-  
using splines. The PI method  
to the following equation.

$$\eta_i \quad (8)$$

case a spline. We have used this  
rogram PIMPLE [14], to screen  
ets, as follows: (i) In a first step,  
prior the model obtained in the  
bility in the PK-PD parameters,  
est fit of all possible products of  
s estimate of  $\eta_i$  for each PK-PD  
e previous NONMEM model is  
rmined in the step (ii). The pair-  
way interactions, and then for  
her improves the fit.

ere found. This suggests that an  
more complex true multivariate  
are often not good enough (too  
s for adequate description.  
nteractions between continuous  
tes and categorical and contin-  
us. For prazosin an interesting  
NONMEM showed that Cl is a  
is a function of HT and HCTZ.  
ng this additive model as a prior  
SEX for both Cl and V. Figure 5  
the Cl of prazosin. Addition of  
SEX to the (additive) NONMEM

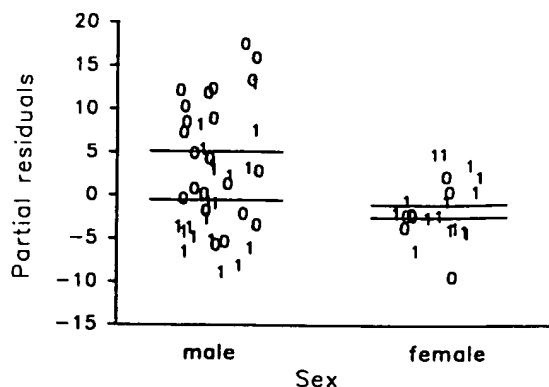


Fig. 5. Partial residual plot showing the interaction between SEX and HCTZ for the clearance of prazosin. The partial residuals are plotted as "0" which indicates no co-treatment with HCTZ or "1" which indicates that the individuals received co-treatment with HCTZ. The solid lines represent the mean partial residual values for each of the four groups.

model results in a significant drop in -2LL of 21.4 on four extra degrees of freedom. This model can be further refined by deleting some of the interaction terms that are not significant and by deleting RACE from the CL model and HT from the V model, resulting, finally, in a model with -2LL 14 points lower than the additive model and with one less parameter. Thus, inclusion of the important interaction term in the NONMEM model results in the removal of two factors, RACE for Cl and HT for V, that appear to be significant in the additive NONMEM model. These two factors are probably substituting for the interaction effect.

## DIAGNOSTICS FOR NONLINEAR MIXED EFFECTS MODELS

The estimation methods in NONMEM (and GAM) can be thought of as extensions of least-squares methods. It is well known that such methods can be very sensitive to "outliers". Thus, model building results can be strongly influenced by only a few influential observations, and the fitted model may reflect the unusual features of those observations rather than the general relationship between covariates and parameters. It is therefore very important to detect those observations that have an unusual large influence on the results of the analysis. Since population analysis focuses on the relationships between individual PK-PD parameters and patient specific covariates, influence is naturally defined at the level of the individual, rather than the level of single observations.

In regression analysis, case-deletion diagnostics are the standard tool used to detect influential observations [15,16]. For a non-linear mixed effects model we propose to use two diagnostics that are generalizations of those used in standard regression analysis: the Cook-score (COOK) and covariance-ratio (COV-ratio). These case-deletion diagnostics, as we use them, measure the influence of the removal of the data of one individual on the parameter estimates of the current model. The overall influence of the deletion of the  $i$ th individual on the estimate of  $\theta$  (the symbol  $\theta$  now is used generically to refer to the vector of all parameters, both fixed effect and random effect, of the population model) can be calculated from the following equations:

$$COOK_i = (\sqrt{(\theta_i - \bar{\theta})^T cov(\bar{\theta})^{-1} (\theta_i - \bar{\theta})}) \quad (9)$$

$$COV - ratio_i = \sqrt{\frac{\det(cov(\theta_i))}{\det(cov(\theta))}} \quad (10)$$

where  $\theta_i$  is the vector of parameter estimates when the  $i$ th individual is deleted,  $cov(.)$  denotes the covariance matrix of the parameter estimates, and  $\det(.)$  denotes the determinant of a matrix. The Cook-score for the  $i$ th individual measures (approximately) the average absolute change in the value of the parameter estimates when the  $i$ th individual's data are deleted, each parameter change scaled to its uncertainty (standard error) when estimated using all the data. The COV-ratio for the  $i$ th individual measures the "overall" uncertainty (standard error) of all parameter estimates on deletion of the  $i$ th individual's data, scaled to the full-data overall uncertainty. A large Cook-score indicates that the individual's data have an inordinate effect on parameter estimate values, and hence are to some degree inconsistent with the rest of the data, while a large value of the COV-ratio indicates that the individual's data contribute considerable information to the fit (are influential). A small value of the Cook-score indicates that the individual's data are compatible with the rest of the data, while a small COV-ratio indicates that parameter precision improves when the individual's data are deleted, suggesting that they are incompatible with the other data.

To detect the influence on a single parameter  $\theta_k$  of the model, similar diagnostics can be calculated using the following equations

$$COOK_{\theta_{k,i}} = \sqrt{\frac{(\theta_{k,i} - \theta_k)^2}{var(\theta_k)}} \quad (11)$$

$$COV - ratio_{\theta_{k,i}} = \sqrt{\frac{var(\theta_{k,i})}{var(\theta_k)}} \quad (12)$$

where  $var(.)$  denotes the variance of the parameter estimate, and  $\theta_{k,i}$  denotes the estimate of  $\theta_k$  when the data of the  $i$ th individual are deleted. The Cook-score for a single parameter and  $i$ th individual measures the change in the value of the parameter estimate when the  $i$ th individual is deleted, scaled to the uncertainty (standard error) when the parameter is estimated using all the data. The COV-ratio for a single parameter and  $i$ th individual measures the uncertainty (standard error) of the parameter estimate on deletion of the  $i$ th individual's data, scaled to the uncertainty when estimated from the full-data. The implications of large and small values of these individual parameter diagnostics for individual subjects' data are the same as for the overall diagnostics, except that they are specific for the particular parameter.

The diagnostics presented here are not intended to provide rules for the rejection of data. Influential subjects' data should not necessarily be eliminated from the analysis. These individuals' data may simply be particularly informative. However, influential subjects may point out weaknesses in the analysis and may suggest that the current model is inadequate or may suggest that additional data need to be collected. For example, if the removal of an influential individual changes the value of an estimated parameter to such an extent that its effect becomes insignificant, one is uncomfortable extrapolating the full-data parameter value to future subjects as its value depends, essentially, on just one individual. In general, influential

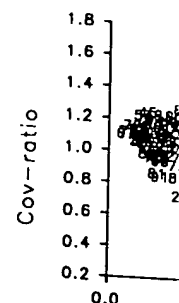


Fig. 6. Plot of the overall Cook-score and the NONMEM model for ibuprofen. The  $n$  individuals (up to 92).

individuals are those whose data (high dimensional) observation-spa with inadequate coverage for reliable analysis goal [15].

In standard regression analysis, case-deletion diagnostics without the effect of each observation (individual). For such methods are currently validated by brute force, i.e., the model developed a NONMEM control stream of the individuals deleted and that estimates and corresponding variance case-deletion diagnostics can then be

## EXAMPLE OF CASE-DELETION I

We here present some results of above to examine the ibuprofen and other 3 drugs.

According to the NONMEM stream WT, DRG and RACE (Eq. 7), and V i

$$V = (\theta_5 + \theta_6(WT - 15) + \theta_7$$

This model was refit to the data with the overall Cook-score and COV-ratio. 6 identifies certain suspect individuals. 84 has a particularly low COV-ratio (influential?), and numbers 31 and 4 both?). These individuals can be further COV-ratio for each of the individuals. plots for the effect of WT ( $\theta_6$ ), LOC (clearly indicate that the data of individual effect on the value of the estimate of example, individual 47's data change

$$\frac{.))}{))}$$

the  $i$ th individual is deleted,  $\hat{\theta}_i$  denotes the parameter estimates, and  $det(.)$  denotes the determinant of the covariance matrix of the individual measures (approximate Fisher information).  $COV_i$  is the ratio of the parameter estimates when the  $i$ th individual is deleted to the change scaled to its uncertainty. The COV-ratio for the  $i$ th individual is the ratio of the determinant of the error (error) of all parameter estimates to the full-data overall uncertainty. The COV-ratio for the  $i$ th individual has an inordinate effect on the degree inconsistent with the rest of the data indicates that the individual's data are influential). A small value of the COV-ratio is compatible with the rest of the data. The COV-ratio improves when the  $i$ th individual is incompatible with the other

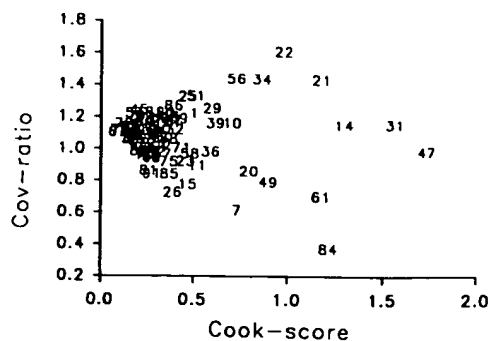
of the model, similar diagnostics

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$$\frac{(c, i)}{(k)}$$

estimate, and  $\theta_{k,i}$  denotes the parameter estimate if the  $i$ th subject is deleted. The Cook-score  $COO_{k,i}$  measures the change in the value of the parameter estimate if the  $i$ th subject is deleted, scaled to the uncertainty in the parameter estimate using all the data. The COV-score  $COV_{k,i}$  measures the uncertainty (standard deviation) in the parameter estimate if the  $i$ th subject is deleted, scaled to the uncertainty in the parameter estimate using all the data. The implications of large and small Cook-scores and COV-scores for individual subjects' data are the same as for the Cook-scores. They are specific for the particular

to provide rules for the rejection necessarily be eliminated from the particularly informative. However, the analysis and may suggest that that additional data need to be that individual changes the value its effect becomes insignificant, parameter value to future subjects individual. In general, influential



**Fig. 6.** Plot of the overall Cook-score and COV-ratio when each of the individuals is deleted from the NONMEM model for ibuprofen. The numbers plotted are the actual index number for each of the individuals (up to 92).

individuals are those whose data are far removed from other individuals in the (high dimensional) observation-space; identification of regions of observation space with inadequate coverage for reliable estimation and prediction is an important data analysis goal [15].

In standard regression analysis, numerical methods are available that calculate case-deletion diagnostics without the need to re-estimate parameters after removal of each observation (individual). For nonlinear mixed effect models, however, no such methods are currently validated and case-deletion diagnostics are best calculated by brute force, i.e., the model is refit with each individual deleted. We have developed a NONMEM control stream that automatically refits the model with each of the individuals deleted and that saves the resulting case-deletion parameter estimates and corresponding variance-covariance matrix of the estimates [17]. The case-deletion diagnostics can then be calculated using Eqs. (9)–(12).

### EXAMPLE OF CASE-DELETION DIAGNOSTICS

We here present some results of using the case-deletion diagnostics described above to examine the ibuprofen analysis, and also some results of so doing for the other 3 drugs.

According to the NONMEM step of the ibuprofen analysis,  $Cl$  is a function of WT, DRG and RACE (Eq. 7), and  $V$  is a function of WT, LOC, DRG, and SEX:

$$V = (\theta_5 + \theta_6(WT - 15) + \theta_7LOC + \theta_8DRG + \theta_9SEX) \cdot \exp(\eta_v) \quad (13)$$

This model was refit to the data with each of the individuals deleted. The values of the overall Cook-score and COV-ratio are shown in Fig. 6 for each individual. Figure 6 identifies certain suspect individuals (those appearing along its “edges”): number 84 has a particularly low COV-ratio (outlier?), number 22 has a high COV-ratio (influential?), and numbers 31 and 47 have high Cook-scores (outlier? influential? both?). These individuals can be further evaluated by plotting the Cook-score vs COV-ratio for each of the individual parameter estimates. Figure 7 shows these plots for the effect of WT ( $\theta_6$ ), LOC ( $\theta_7$ ), DRG ( $\theta_8$ ), and SEX ( $\theta_9$ ) on V. These figures clearly indicate that the data of individuals 31, 84, and 47 have an unusually great effect on the value of the estimate of V when compared to other individuals. For example, individual 47’s data changes  $\theta_9$  almost 1.5 times the standard error of the



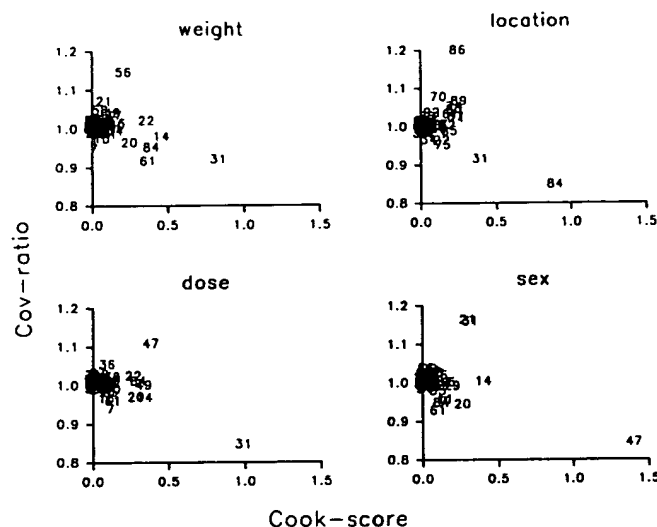


Fig. 7. Plot of the Cook-score and COV-ratio for the parameters describing the effect of WT ( $\theta_6$ ), LOC ( $\theta_7$ ), DRG ( $\theta_8$ ), and SEX ( $\theta_9$ ) on the volume of distribution of ibuprofen. The numbers plotted are the actual index number for each of the individuals (up to 92).

parameter estimate. Table V summarizes the parameter estimates for the model for V when each of these individuals is deleted from the analysis. Removal of individual 31's data renders the effect of DRG insignificant, whereas removal of individual 47's data renders the effect of SEX insignificant. Both effects are highly significant ( $p < 0.01$ ) according to the likelihood ratio test when all individuals are included in the analysis. Furthermore, the magnitude of these (binary) effects in the full-data analysis is notable: both change the average V by about 18%. The number of males/females was 53/40, while the number of subjects with low/high DRG was 45/48. Since all these numbers are appreciable, the fact that the apparent effects of the covariates change so profoundly upon deletion of just one individual in each case suggests that the "covariate effect" is not real, but simply an artefact due to outlying individuals. If, however, there had been severe covariate imbalance; i.e., only a few individuals with the same SEX value as number 47, or only a few individuals with the same DRG value as number 31, then one might suspect that the effect could be real, and seek confirmation through study of additional individuals with like covariate values. The effect of removal of the data of individual 84 on  $\theta_7$  is also profound. LOC is a highly significant factor when the data of all individuals are analyzed, changing  $-2LL$  by 50 points when included in the model. Yet one must conclude that the magnitude of its effect is poorly defined, since it changes by almost 1 standard error when subject 84 is removed. In all but the last of the above cases, it is true that the standard errors of the parameter estimates based on the full data are sufficiently large that the covariate effects are suspect, but the case-deletion diagnostics add considerable specificity to this vague suspicion.

Case-deletion diagnostics will identify influential individuals even though they be otherwise difficult to identify due to the complexity of the population data. Even may plots of the empirical Bayes estimates of the model parameters vs the covariates such as in Fig. 2 may not clearly identify influential subjects because of the complex influence structure of each subject on all parameter estimates.

Case-deletion diagnostics are also helpful in explaining some of the prazosin results. For this drug, several covariates are found to significantly influence CI (see Table IV) and V, resulting in a marked drop in  $-2LL$  when they are included. However,

Table V. Effect of removal of certain estimates of the NONMEM population compared to the parameter figures in each cell are the parameter in  $-2LL$  (based on the analysis with set to zero is also shown (first col

|                        | $-2\Delta LL$<br>(vs. $\theta=0$ ) |
|------------------------|------------------------------------|
| $\theta_5$             |                                    |
| $\theta_6(\text{wt})$  | 77.5                               |
| $\theta_7(\text{loc})$ | 50.2                               |
| $\theta_8(\text{drg})$ | 8.3                                |
| $\theta_9(\text{sex})$ | 14.4                               |
| $\omega_{V2}$          |                                    |

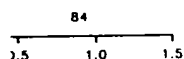
the inter-individual variability in explanatory covariates, a people variability is being "explained", and deletion diagnostics reveal that this results in a 25% decrease of inter-standard error of this estimate. The individual variability in V. This suggests compared to other subjects. How and V were not well explained by

## CONCLUSIONS

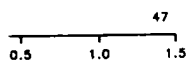
In this paper we have shown an approach to selecting a good initial model. The GAM step selects the most important linearities in the relationships between the covariates and the response. This method is efficient and provides insight into the structure of the GAM. In this paper we here present a screening method for our four data sets, no interactions were found. An adequate approximation to an infinite surface.

We have also shown that case-deletion data is unusually influential in the model. This should allow population modeling.

location



sex



describing the effect of WT ( $\theta_6$ ), LOC  
ofen. The numbers plotted are the

parameter estimates for the model  
in the analysis. Removal of  
significant, whereas removal of  
constant. Both effects are highly  
tested when all individuals are  
one of these (binary) effects in  
parameter V by about 18%. The  
number of subjects with low/high  
ble, the fact that the apparent  
deletion of just one individual  
is real, but simply an artefact  
of severe covariate imbalance;  
as number 47, or only a few  
when one might suspect that the  
study of additional individuals  
the data of individual 84 on  $\theta_7$   
when the data of all individuals  
included in the model. Yet one  
is only defined, since it changes by  
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parameter estimates based on the full  
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individuals even though they  
of the population data. Even  
parameters vs the covariates  
objects because of the complex  
estimates.

explaining some of the prazosin  
significantly influence CI (see  
when they are included. However,

**Table V.** Effect of removal of certain individuals' data (#31, #47, #84) on the parameter estimates of the NONMEM population model for the volume of distribution of ibuprofen compared to the parameter estimates based on all individuals' data (all #). The figures in each cell are the parameter estimate (standard error of estimate). The change in  $-2LL$  (based on the analysis with all #) when each of the parameters estimates ( $\theta$ ) is set to zero is also shown (first column).

|                  | $-2\Delta LL$<br>(vs. $\theta=0$ ) | all #            | - #31            | - #47            | - #84            |
|------------------|------------------------------------|------------------|------------------|------------------|------------------|
| $\theta_5$       |                                    | 2.12<br>(0.16)   | 2.28<br>(0.16)   | 2.19<br>(0.11)   | 2.21<br>(0.14)   |
| $\theta_6$ (wt)  | 77.5                               | 0.111<br>(0.015) | 0.099<br>(0.014) | 0.109<br>(0.016) | 0.117<br>(0.014) |
| $\theta_7$ (loc) | 50.2                               | 1.91<br>(0.47)   | 1.73<br>(0.42)   | 1.96<br>(0.46)   | 1.50<br>(0.39)   |
| $\theta_8$ (drg) | 8.3                                | 0.38<br>(0.22)   | 0.16<br>(0.19)   | 0.30<br>(0.24)   | 0.31<br>(0.22)   |
| $\theta_9$ (sex) | 14.4                               | 0.37<br>(0.15)   | 0.33<br>(0.18)   | 0.15<br>(0.13)   | 0.35<br>(0.22)   |
| $\omega_{V2}$    |                                    | 0.083<br>(0.029) | 0.094<br>(0.030) | 0.087<br>(0.029) | 0.072<br>(0.025) |

the inter-individual variability in CI and V is not reduced by inclusion of these explanatory covariates, a perplexing contradiction (unexplained inter-individual variability is being "explained", why then does its magnitude not decrease?). Case-deletion diagnostics reveal that this is due to one subject. Removal of this subject results in a 25% decrease of inter-individual variance in CI and a 50% decrease in the standard error of this estimate. Similar results are found for the estimate of inter-individual variability in V. This subject did not have unusual CI and V estimates when compared to other subjects. However, in contrast to the others, this individual's CI and V were not well explained by the final population model.

## CONCLUSIONS

In this paper we have shown that a preliminary GAM analysis is a robust approach to selecting a good initial model for a subsequent NONMEM analysis. The GAM step selects the most important covariates and indicates important non-linearities in the relationships between covariates and PK-PD model parameters. The method is efficient and provides illustrative graphics. A disadvantage of the additive structure of the GAM is that interactions between covariates are not included, but we here present a screening method to detect such interactions. When it is applied to our four data sets, no interactions are found, suggesting that a GAM may often be an adequate approximation to an inevitably more complex true multivariate regression surface.

We have also shown that case-deletion diagnostics can detect individuals whose data is unusually influential in the final NONMEM analysis. Use of such diagnostics should allow population modeling to become more robust.

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